JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Structure Elucidation of New Fusarins Revealing Insights in the Rearrangement Mechanisms of the *Fusarium* Mycotoxin Fusarin C

Karin Kleigrewe,[†] Filiz Aydin,[†] Kirstin Hogrefe,[†] Patrick Piecuch,[†] Klaus Bergander,[‡] Ernst-Ulrich Würthwein,[‡] and Hans-Ulrich Humpf*[,†]

[†]Institute of Food Chemistry and [‡]Organic Chemistry Institute, Westfälische Wilhelms-Universität Münster, Münster, Germany

Supporting Information

ABSTRACT: Fusarin C is a *Fusarium* mycotoxin that rearranges under reversed phase chromatographic conditions. In this study, the rearrangement of fusarin C was examined in detail, and the formation of fusarins under different conditions was optimized. All relevant fusarins including (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C were isolated and identified by NMR. To confirm the involvement of the 2-pyrrolidone ring in the rearrangement of fusarin C, 15-methoxy-fusarin C was synthesized. For the first time, the structure of open-chain fusarin C was elucidated, and on the basis of these data, the rearrangement product of fusarin C was identified as *epi*-fusarin C. The results were confirmed by detailed NMR measurements and density functional theory calculations. Furthermore, a new fusarin C like metabolite, which was named dihydrofusarin C, was detected by analysis of the crude extract of fusarin C with high-performance liquid chromatography coupled to UV and Fourier transform mass spectrometry.

KEYWORDS: *fusarin C, Fusarium, metabolite, mycotoxin, Fourier transform mass spectrometry, rearrangement, nuclear magnetic resonance, open-chain fusarin C, epi-fusarin C, dihydrofusarin C, density functional theory*

INTRODUCTION

Fusarin C is a *Fusarium* mycotoxin that occurs in food and feed[1−4] and is mutagenic after metabolic activation.[3,5−8] It induces chromosomal aberrations, micronuclei, sister chromatid exchange, and the formation of 6-thioguanine-resistant mutants in V79 cells as well as asynchronous replication of polyoma DNA sequences after the presence of a microsomal activation system.[6,8] Nevertheless, toxicological studies with fusarin C are quite challenging because of the light-induced rearrangement of fusarin C and the co-occurrence of further fusarin C analogues. In previous studies, little or no information was provided on the purity of the isolated fusarin C, or even mixtures of unknown fusarins were used. In addition, crude fusarin extracts have been shown to be more mutagenic than pure fusarin C.[8]

Figure 1 illustrates fusarin C and different analogues. All fusarins have a 2-pyrrolidone ring in common, as well as a pentaene chain.[5,7] The pentaene chain can easily rearrange under UV light, forming several stereoisomers like (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C.[9−12] Furthermore, an intramolecular ring closure of the 2-hydroxyethyl side chain leads to the formation of fusarins D and A.[12,13] A recent report investigated the stability of fusarins A and C in a buffer system and suggested that these fusarins rearrange.[14] Savard and Miller[13] also reported a rearrangement of fusarin C under reversed phase (RP) conditions and postulated the structure of fusarin F. This rearrangement was assigned to a migration of the epoxide group at the 2-pyrrolidone ring from C-13/C-14 to C14/C-15[13] (Figure 1). The structure of fusarin F was assigned based on ^{1}H and ^{13}C NMR data. However, the authors did not discuss another possible epimerization of the 15-OH group, which was postulated for lucilactaene, a similar compound.[15]

Therefore, we conducted studies to elucidate the rearrangement of fusarin C. First, analogues of fusarin C found in cultures as well as after irradiation with UV light were isolated and analyzed by Fourier transform mass spectrometry (FTMS) and NMR. To verify that the 2-pyrrolidone ring takes part in the rearrangement of fusarin C, a 15-*O*-protected fusarin C was synthesized. Furthermore, NMR data of possible rearrangement products were calculated by density functional theory (DFT) methods and compared to the experimental results. On the basis of these data, a new rearrangement mechanism for fusarin C was proposed, which could finally be proven experimentally by the isolation and structure elucidation of a new fusarin C analogue formed as an intermediate during the rearrangement of fusarin C.

MATERIALS AND METHODS

Chemicals and Materials. All chemicals were purchased from Sigma-Aldrich GmbH (Seelze, Germany), Carl Roth GmbH + Co. KG (Karlsruhe, Germany), or VWR International GmbH (Darmstadt, Germany). Solvents were obtained in gradient grade quality. Water for high-performance liquid chromatography (HPLC) separation was purified by a Milli-Q Gradient A 10 system (Millipore, Schwalbach, Germany).

Isolation of Fusarins. The strain *F. verticillioides* MRC0712 was used for the production of fusarins. A battery of 10 Erlenmeyer flasks (250 mL), containing 100 mL of MYRO-media[2] each, were inoculated with mycelia (ca. 0.5 cm^2) grown on CM agar.[16] The cultures were incubated for 5 days on a rotary shaker at 28 °C and 70 rpm in the absence of light. The mycelia were removed from the culture fluids by filtration through Miracloth (Calbiochem, Merck KGaA, Darmstadt). To prevent plugging of the solid-phase extraction (SPE) cartridge used

Received: March 4, 2012
Revised: April 26, 2012
Accepted: April 28, 2012
Published: April 28, 2012

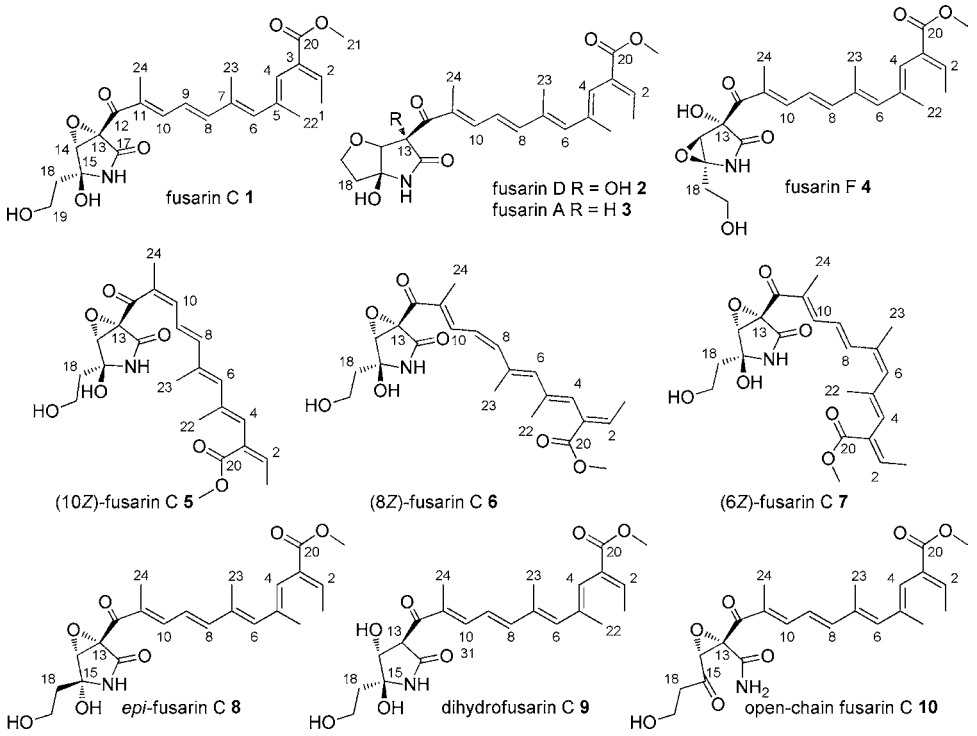

Figure 1. Structures of fusarins.

later, three additional filtration steps with filter paper MG 615 (Macherey-Nagel GmbH, Düren, Germany) were performed. The culture filtrates were extracted by SPE with C18 material. A 10 g Strata C-18E, 55 μm, 70A Giga Tubes SPE column (Phenomenex, Aschaffenburg) was conditioned with 50 mL of MeOH and 50 mL of H_2O, respectively. Afterward, approximately 1 L of culture filtrate was added to the column. To remove salts and sugars from the media, the column was washed with 100 mL of H_2O. The fusarin fraction was eluted with MeOH/H_2O (70/30, v/v). The solvent was removed by a rotary evaporator, and the crude extract was redissolved either in CH_2Cl_2/MeOH (95/5, v/v) or in MeOH/H_2O (50/50, v/v) and further purified by preparative HPLC.

Purification of Fusarins with Normal Phase (NP) Preparative HPLC. The crude extract obtained after SPE cleanup was separated on a 250 mm × 10 mm i.d., 5 μm, LiChrospher Si 60 column (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) using isocratic conditions. A Jasco PU-2089 low-pressure gradient HPLC pump (Jasco, Gross-Umstadt, Germany) coupled to a Jasco diode array detector (MD-2010 Plus) was used. The solvent composition was 95% CH_2Cl_2 and 5% MeOH (v/v), the flow rate was set to 5 mL/min, and the runtime was 15 min. Data were recorded in a wavelength range of 200−650 nm, and the extracted wavelength of 363 nm was used for identifying the fractions containing fusarins. Acquisition of data was carried out with Chrompass, Chromatography Data system (Version 1.8.6.1, Jasco).

To prevent an isomerization of fusarin C and its analogues, the fractions were collected in deactivated glassware. The solvent was removed by a rotary evaporator. One batch of cultivation yielded 15−20 mg of fusarin C (7.5 min), 10−15 mg of *epi*-fusarin C (8.7 min), and 5−10 mg of fusarin D (9.9 min). Further fusarin C analogues like fusarin A (3.6 mg, 3.9 min) and open-chain fusarin C (3.8 mg, 4.3 min) were isolated from multiple batches.

Deactivation of Glassware. A 5−10% solution of dimethyldichlorosilane in toluene was used for the deactivation. Glassware was filled with the reagent for 30 min. Afterward, the glassware was washed with toluene and methanol, respectively.

Purification of Fusarins with RP Preparative HPLC. The crude extract obtained after SPE cleanup was separated on a 250 mm × 9 mm i.d., 5 μm, Agilent Eclipse XDB C-18 column (Agilent Technologies, Böblingen, Germany) using isocratic conditions. A Jasco PU-2087 high-pressure gradient HPLC pump coupled to a Jasco UV-2075 UV/vis detector was used (Jasco, Gross-Umstadt, Germany). The solvent composition was 58% MeOH/tetrahydrofuran (95/5, v/v) and 42% water, the flow rate was set to 3 mL/min, and the runtime was 50 min. The wavelength was set to 363 nm. Acquisition of data was carried out with Jasco-Borwin software (Version 1.5, Jasco). Dihydrofusarin C eluted at 20 min, *epi*-fusarin C at 24 min, fusarin C at 27 min, and fusarin D at 30 min. Two milligrams of dihydrofusarin C was obtained.

Analytical HPLC-DAD of Fusarins. To monitor the rearrangement of fusarins, samples were analyzed by analytical HPLC either with RP or with NP chromatography. RP chromatography was carried out according to our previous publication.[1] For NP chromatography, a 250 mm × 4 mm i.d., 5 μm, LiChrospher Si 60 column (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) was used with the same HPLC system as described above. The flow rate was set to 1 mL/min, and the solvent composition contained 95% CH_2Cl_2 and 5% MeOH (v/v). The wavelength range of 200−650 nm was recorded, and chromatograms were monitored at a wavelength of 363 nm.

HPLC-UV-FTMS. Exact mass measurements and fragmentation experiments were carried out by HPLC with FTMS detection according to our previous publication.[1] To analyze the fragmentation pattern of open-chain fusarin C, a NP HPLC system was used. Therefore, an Accela LC 60057-60010 system (Thermo Fisher Scientific, Bremen, Germany) was linked to a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Data acquisition was performed with Xcalibur 2.07 SP 1 (Thermo Fisher Scientific). Chromatographic separation was performed on a 250 mm × 4 mm i.d., 5 μm, LiChrospher Si 60 column. The injection volume was 10 μL, and the flow rate was 1 mL/min. The HPLC was programmed isocratically with 95% CH_2Cl_2 and 5% MeOH (v/v). Before entering the mass spectrometer, the eluate was split at a ratio of 1:2. The mass spectrometer was operated in the positive ionization mode, and ionization was performed with heated electrospray ionization with the same parameters as described previously.[1]

For analysis of crude extracts, a Knauer variable wavelength detector (Knauer, Berlin, Germany) was coupled additionally to the FTMS. The wavelength was set to 363 nm.

Table 1. ^{13}C NMR of Fusarins

C-atom[a]	fusarin C	*epi*-fusarin C	open chain	fusarin D	fusarin A	dihydro fusarin C	(10*Z*)-fusarin C	(8*Z*)-fusarin C	(6*Z*)-fusarin C	15-methoxy-fusarin C
1	16.4	16.3	16.4	16.3	16.3	16.3	16.3	16.6	16.1	16.3
2	140.6	140.6	140.6	140.4	140.6	140.4	140.4	141.8	140.7	140.5
3	130.9	130.9	130.9	131.0	131.0	130.6	130.3	130.8	130.7	130.9
4	126.9	127.0	127.0	126.4	126.9	126.4	126.2	124.5	125.2	126.8
5	138.0	138.0	137.9	138.0	137.9	137.6	138.0	139.7	137.5	137.9
6	141.3	141.4	141.5	140.2	141.3	140.8	139.5	136.9	138.4	141.0
7	135.6	135.6	135.5	135.7	135.6	135.3	135.8	134.3	134.4	135.6
8	149.6	149.5	149.4	147.9	149.4	149.6	147.8	144.4	141.7	149.1
9	124.0	123.9	123.7	124.2	124.2	124.2	125.5	125.0	125.0	124.1
10	146.4	146.3	145.2	144.3	146.5	145.8	143.3	140.8	145.4	146.2
11	134.0	134.0	132.6	133.2	134.5	135.6	131.0	135.2	134.2	134.2
12	190.7	190.1	191.2	197.6	197.8	198.3	190.7	190.1	189.7	190.2
13	62.3	64.7	66.7	85.7	57.2	53.4	63.1	62.7	61.8	62.2
14	64.3	63.2	63.2	91.9	86.3	74.3	63.7	64.6	64.4	63.1
15	86.0	85.3	202.7	95.7	95.0	86.6	85.5	86.2	85.6	88.8
17	170.5	168.7	166.5	175.8	171.0	Na	169.2	169.9	169.9	170.3
18	36.4	39.8	44.4	38.6	38.0	39.5	31.8	36.9	36.6	36.6
19	58.8	58.1	57.8	69.3	69.1	58.6	60.0	59.3	58.6	58.4
20	168.0	168.0	167.9	168.0	167.6	167.7	168.0	169.7	168.4	167.9
21	52.3	52.3	52.3	52.2	52.3	51.7	52.2	52.8	52.4	52.2
22	19.1	19.1	19.1	19.1	19.1	19.0	19.1	19.2	18.8	19.1
23	14.4	14.5	14.5	14.5	14.5	14.4	14.6	19.6	20.7	14.5
24	11.7	11.7	11.9	12.8	11.9	11.5	20.2	11.5	11.4	11.6
25										50.6

[a]For a better comparison of the NMR data, the same numbering was used for all compounds (see Figure 1). Chemical shifts are reported in ppm, and all samples were measured in CD_2Cl_2.

Spectroscopic and Spectrometric Data. NMR data of main fusarins are summarized in Tables 1 and 2.

Fusarin C. ESI-MS (positive mode): FTMS *m/z* 454.1834 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: *m/z* 454.1836). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 290 (100), 335 (51), 436 (34), 406 (23), 368 (23), 352 (18), 267 (14), 396 (13), 419 (13), 408 (11), 341 (11), 426 (10). $[\alpha]_D^{23} = 47°$ (*c* 4.68 mg/mL, MeOH).

epi-Fusarin C. ESI-MS (positive mode): FTMS *m/z* 454.1835 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: *m/z* 454.1836). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 368 (100), 290 (50), 227 (33), 268 (10), 295 (9), 325 (9). $[\alpha]_D^{23} = 15°$ (*c* 5.99 mg/mL, MeOH).

Open-Chain Fusarin C. ESI-MS (positive mode): FTMS *m/z* 432.2017 (calculated mass for $[C_{23}H_{29}O_7N + H]^+$: *m/z* 432.2017). MS/MS (CID 35%; $[M + H]^+$): *m/z* (%): 414 (100), 396 (49), 400 (36), 382 (23), 364 (14), 369 (6), 295 (4). FTMS *m/z* 454.1837 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: *m/z* 454.1836). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 290 (100), 368 (63), 419 (12), 436 (12), 436 (12). $[\alpha]_D^{23} = -9°$ (*c* 1.27 mg/mL, MeOH).

Fusarin D. ESI-MS (positive mode): FTMS *m/z* 454.1835 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: *m/z* 454.1836). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 368 (100), 290 (48), 227 (32), 436 (11), 268 (10), 295 (10), 325 (10), 269 (8). $[\alpha]_D^{23} = -65°$ (*c* 3.63 mg/mL, MeOH).

Fusarin A. ESI-MS (positive mode): FTMS *m/z* 438.1888 (calculated mass for $[C_{23}H_{29}O_6N + Na]^+$: *m/z* 438.1887). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 274 (100), 420 (46), 269 (17), 295 (13), 410 (5), 222 (5). $[\alpha]_D^{23} = -23°$ (*c* 1.06 mg/mL, MeOH).

Dihydrofusarin C. ESI-MS (positive mode): FTMS *m/z* 456.1991 (calculated mass for $[C_{23}H_{31}O_7N + Na]^+$: *m/z* 456.1993). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 438 (100), 420 (83), 269 (56), 406 (35), 408 (23), 292 (19), 337 (17).

epi-Dihydrofusarin C. ESI-MS (positive mode): FTMS *m/z* 456.1993 (calculated mass for $[C_{23}H_{31}O_7N + Na]^+$: *m/z* 456.1993). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 437 (100), 354 (11), 292 (3), 201 (2). ^1H NMR (600 MHz, CD_2Cl_2): δ 7.48 (d, 1, *J* = 10.2 Hz), 6.96 (ddd, 1, *J* = 7.2, 6.7, 1.0 Hz), 6.89 (d, 1, *J* = 15.1 Hz), 6.68 (dd, 1, *J* = 15.0, 11.0 Hz), 6.57 (s, 1), 6.34 (s, 1), 6.09 (s, 1), 4.60 (d, 1, *J* = 8.1 Hz), 4.42−4.37 (m, 1), 4.03−3.97 (m, 1), 3.95−3.86 (m, 1), 3.72 (s, 3), 2.11 (d, 3, *J* = 1.0 Hz), 1.99 (s, 3), 1.78 (ddd, 3, *J* = 7.2, 2.5, 1.4 Hz), 1.74 (d, 3, *J* = 1.3 Hz).

Synthesis of 15-Methoxy-fusarin C and 15-Methoxy-dihydrofusarin C. To demonstrate that the 15-hydroxy group is involved in the rearrangement of fusarin C, this group was protected stereoselectively according to a procedure already published for similar compounds.[15] The reaction was monitored with thin-layer chromatography according to Barrero et al.,[9] and the reaction mixture purified by preparative HPLC under RP conditions on a 250 mm × 9 mm i.d., 5 μm, Agilent Eclipse XDB-C18 column using isocratic conditions. A Jasco PU-2087 HPLC pump coupled to a Jasco UV-2075 UV/vis detector was used (Jasco, Gross-Umstadt, Germany). The solvent composition was 59% MeOH/tetrahydrofuran (95/5, v/v) and 41% H_2O, the flow rate was set to 3 mL/min, and the runtime was 60 min. The wavelength was set to 363 nm. Acquisition of data was carried out with Borwin (Version 1.5, Jasco). As dihydrofusarin C epimerizes like fusarin C, the 15-hydroxy group of dihydrofusarin C was also protected in the same way.

15-Methoxy-fusarin C. ESI-MS (positive mode): FTMS *m/z* 468.1990 (calculated mass for $[C_{24}H_{31}O_7N + Na]^+$: *m/z* 468.1993). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 304 (100), 406 (98), 440 (61), 410 (57), 436 (51), 418 (46), 382 (31), 335 (29), 264 (23), 438 (19), 323 (16), 378 (15). For NMR data, see Tables 1 and 2.

15-Methoxy-dihydrofusarin C. ESI-MS (positive mode): FTMS *m/z* 470.2145 (calculated mass for $[C_{24}H_{33}O_7N + Na]^+$: *m/z* 470.2149). MS/MS (CID 35%; $[M + Na]^+$): *m/z* (%): 438 (100), 408 (65), 420 (60), 452 (48), 269 (35), 337 (15), 306 (15). ^1H NMR (600 MHz, CD_2Cl_2): δ 7.25 (dd, J = 11.1, 1.3 Hz, 1H), 6.96 (qd, *J* = 7.5, 1.3 Hz, 1H), 6.79 (d, *J* = 15.3 Hz, 1H), 6.69 (dd, *J* = 15.0, 10.6 Hz, 1H), 6.33 (s, 1H), 6.27 (s, 1H), 6.06 (s, 1H), 4.71 (t, *J* = 8.5 Hz, 1H), 4.33 (d, *J* = 8.8 Hz, 1H), 3.82 (dd, *J* = 11.0, 4.9 Hz, 2H), 3.71 (s, 3H), 3.36 (s, *J* = 4.3 Hz, 3H), 2.10 (d, *J* = 1.0 Hz, 3H), 2.08−2.00 (m, 2H), 2.00 (d, *J* = 0.9 Hz, 3H), 1.78 (dd, *J* = 7.2, 1.4 Hz, 3H), 1.73 (d, *J* = 1.2 Hz, 3H).

Table 2. ^{1}H NMR of Fusarins

C[a]	fusarin C	*epi*-fusarin C	open chain	fusarin D	fusarin A	dihydrofusarin C	(10Z)-fusarin C	(8Z)-fusarin C	(6Z)-fusarin C	15-methoxy-fusarin C
1	1.77 (dd, 3, 7.2, 1.1)	1.77 (dd, 3, 7.2, 1.4)	1.78 (dd, 3, 7.2, 1.4)	1.76 (d, 3, 7.2)	1.78 (dd, 3, 7.2, 1.4)	1.78 (ddd, 3, 7.2, 2.5, 1.4)	1.76 (dd, 3, 7.2, 1.3)	1.81 (dd, 3, 7.2, 1.3)	1.78 (dd, 3, 7.2, 1.4)	1.77 (dd, 3, 7.2, 1.4)
2	6.96 (qd, 1, 7.2, 1.4)	6.96 (qd, 1, 7.3, 1.2)	6.96 (qd, 1, 7.1, 0.9)	6.95 (q, 1, 7.3)	6.96 (qd, 1, 7.2, 1.0)	6.97 (ddd, 1, 7.2, 6.7, 1.0)	6.94 (q, 1, 7.2)	7.02 (qd, 1, 7.2, 0.9)	6.97 (qd, 1, 7.2, 1.1)	6.96 (ddd, 1, 14.4, 7.2, 1.1)
4	6.07 (s, 1)	6.08 (s, 1)	6.09 (s, 1)	6.05 (s, 1)	6.09 (s, 1)	6.06 (s, 1)	6.03 (s, 1)	6.02 (s, 1)	5.90 (s, 1)	6.08 (s, 1)
6	6.31 (s, 1)	6.32 (s, 1)	6.33 (s, 1)	6.26 (s, 1)	6.33 (s, 1)	6.27 (s, 1)	6.22 (s, 1)	6.02 (s, 1)	6.18 (s, 1)	6.30 (s, 1)
8	6.81 (d, 1, 15.0)	6.81 (d, 1, 14.8)	6.83 (d, 1, 15.0)	6.75 (d, 1, 15.2)	6.88 (d, 1, 14.9)	6.78 (d, 1, 15.4)	6.59 (d, 1, 14.8)	6.39 (d, 1, 11.3)	7.46 (d, 1, 15.3)	6.79 (d, 1, 15.1)
9	6.66 (dd, 1, 15.0, 11.0)	6.67 (dd, 1, 15.0; 11.2)	6.65 (dd, 1, 15.0, 11.0)	6.62 (dd, 1, 15.3, 11.4)	6.68 (dd, 1, 15.0, 11.0)	6.68 (dd, 1, 15.0, 11.0)	7.23 (dd, 1, 15.0, 11.6)	6.46 (q, 1, 12.4)	6.71 (dd, 1, 14.9, 10.9)	6.68 (dd, 1, 14.9, 11.0)
10	7.51 (d, 1, 11.4)	7.42 (d, 1, 11.3)	7.40 (d, 1, 11.4)	7.40 (d, 1, 12.2)	7.51 (d, 1, 11.0)	7.29 (d, 1, 10.8)	6.56 (d, 1, 9.8)	7.66 (d, 1, 10.7)	7.46 (d, 1, 11.0)	7.49 (d, 1, 11.1)
13					4.35 (s, 1)	4.64 (d, 1, 9.1)				
14	4.05 (d, 1, 2.5)	4.14 (d, 1, 2.3)	4.11 (s, 1)	4.33 (s, 1)	4.22 (s, 1)	4.36–4.33 (m, 1)	4.10 (d, 1, 2.4)	3.98 (d, 1, 2.4)	4.04 (d, 1, 2.4)	3.95 (d, 1, 2.6)
16	7.04 (d, 1H, 2.4)	6.32 (s, 1)	6.54 (s, 1)/ 5.74 (s, 1)	7.97 (s, 1)	6.33 (s, 1)	6.57 (s,1)	6.27 (s, 1)	7.20 (s, 1)	6.65 (s, 1)	6.33 (s, 1)
18	2.13–2.10 (m, 1)/ 2.08–2.04 (m, 1)	2.19–2.15 (m, 1)/ 2.14–2.11 (m, 1)	2.86 (t, 2, 5.5)	2.27 (t, 2, 7.7 H)	2.42–2.31 (m, 1)/ 2.32–2.21 (m, 1)	2.06–2.00 (m, 1)/ 1.95–1.87 (m, 1)	2.34–2.25 (m, 1)/ 2.13–2.06 (m, 1)	2.13–2.06 (m, 2)	2.09–2.04 (m, 2)	2.07–2.04 (m, 2)
19	4.05–4.02 (m, 1)/ 3.95–3.89 (m, 1)	3.99–3.89 (m, 2)	3.98–3.85 (m, 2)	3.99–3.93 (m, 1)/ 3.81–3.73 (m, 1)	4.14–4.06 (m, 1)/ 4.03–3.97 (m, 1)	4.03–3.97 (m, 1)/ 3.95–3.86 (m, 1)	4.25–4.15 (m, 1)/ 4.14–4.03 (m, 1)	4.15–4.09 (m, 1)/ 3.98–3.92 (m, 1)	4.10–4.05 (m, 1)/ 3.97–3.88 (m, 1)	4.01–3.96 (m, 1)/ 3.86–3.80 (m, 1)
21	3.71 (s, 3)	3.71 (s, 3)	3.72 (s, 3)	3.71 (s, 3)	3.72 (s, 3)	3.71 (s, 3)	3.71 (s, 3)	3.77 (s, 3)	3.77 (s, 3)	3,71 (s, 3)
22	1.73 (d, 3, 1.2)	1.74 (d, 3, 1.2)	1.74 (d, 3,1.2)	1.72 (s, 3)	1.74 (d, 3, 1.1)	1.73 (d, 3, 1.1)	1.71 (s, 3)	1.70 (d, 3, 1.0)	1.63 (d, 3, 0.8)	1,73 (d, 3, 1,2)
23	2.09 (d, 3, 0.9)	2.10 (d, 3, 1.0)	2.09 (s, 3)	2.08 (s, 3)	2.11 (d, 3, 0.9)	2.11 (d, 3, 1.0)	2.05 (s, 3)	2.00 (s, 3)	2.02 (d, 3, 1.3)	2.10 (d, 3, 1.1)
24	1.98 (d, 3, 1.1)	1.97 (d, 3, 1.0)	1.96 (s, 3)	1.97 (s, 3)	1.97 (d, 3, 0.7)	1.99 (s, 3)	2.14 (s, 3)	1.96 (s, 3)	1.98 (d, 3, 1.0)	1.97 (d, 3, 1.2)
25										3.41 (s, 3)

[a]For a better comparison of the NMR data, the same numbering was used for all compounds (see Figure 1 for structures). Chemical shifts are reported in ppm. All samples were measured in CD_2Cl_2. Parentheses contain signal multiplicity, number of protons, and coupling constants in Hz, respectively.

dx.doi.org/10.1021/jf3009469 | J. Agric. Food Chem. 2012, 60, 5497–5505

Quantum Chemical Calculations. High-level DFT calculations were used to compare experimental NMR data of fusarin C, *epi*-fusarin C, and fusarin F with calculated chemical shifts. First, the structures of fusarin C, fusarin F, and *epi*-fusarin C were simplified as shown in Figure 3. Because the hydroxy groups of the C-19 and C-15 atoms can form hydrogen bonds with the oxygen of the epoxide group, the keto group of the C-17, and the nitrogen atom, several conformers with different hydrogen bonding were optimized using PCModel (V9.1, Serena-Software, Bloomington, IN). To reduce the computation time for the DFT calculations, the geometry for each molecule was first optimized semiempirically using the PM6 method[17] as implemented in the MOPAC 2009[18] program. Using these semiempirical structures, DFT computations were performed using the Gaussian 03 suite of programs. The Becke three-parameter exchange functional and the correlation functional of Lee, Yang, and Parr (B3LYP) with the 6-311+G(d,p) basis set were used to optimize the geometries of the structures and subsequently to calculate their NMR data (NMR shielding tensors and spin−spin coupling constants) by applying the gauge-independent atomic orbital (GIAO) method.[19] All stationary points were verified by frequency analyses.

Isolation of (*Z*)-Isomers. The pentaene chain of fusarin C can easily rearrange to form several isomers like (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C. To induce those structures, a solution of fusarin C (c = 15 mg/mL) was irradiated at a wavelength of 366 nm for about 4−6 h with a CabUVIS UV-analyzing lamp (DESAGA GmbH, Wiesloch, Germany). The rearrangement was monitored by HPLC-DAD.

As soon as an equilibrium was reached, the (*Z*)-isomers were separated with NP preparative HPLC as described above. A total amount of 60 mg of fusarin C yielded 4.2 mg of (6*Z*)-fusarin C, 8.3 mg of (8*Z*)-fusarin C, and 8.1 mg of (10*Z*)-fusarin C. NMR data are summarized in Tables 1 and 2.

(6Z)-Fusarin C. ESI-MS (positive mode): FTMS m/z 454.1836 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: m/z 454.1836). MS/MS (CID 35%; $[M + Na]^+$): m/z (%): 335 (100), 436 (48), 267 (41), 406 (28), 352 (26), 290 (22), 418 (17), 388 (11), 239 (10). $[\alpha]_D^{23}$ = 18° (c 1.25 mg/mL, MeOH).

(8Z)-Fusarin C. ESI-MS (positive mode): FTMS m/z 454.1836 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: m/z 454.1836). MS/MS (CID 35%; $[M + Na]^+$): m/z (%): 335 (100), 436 (51), 267 (39), 352 (28), 406 (28), 290 (24), 418 (15), 426 (11). $[\alpha]_D^{23}$ = −127° (c 2.33 mg/mL, MeOH).

(10Z)-Fusarin C. ESI-MS (positive mode): FTMS m/z 454.1834 (calculated mass for $[C_{23}H_{29}O_7N + Na]^+$: m/z 454.1836). MS/MS (CID 35%; $[M + Na]^+$): m/z (%): 419 (100), 436 (86), 335 (83), 267 (52), 406 (36), 352 (33), 418 (31), 408 (16), 388 (13), 393 (10), 391 (10), 290 (9). $[\alpha]_D^{23}$ = 30° (c 2.46 mg/mL, MeOH).

NMR Spectroscopy. The ^1H, ^{13}C, and 2D NMR spectra were acquired either on a Bruker DPX-400 (Bruker BioSpin, Rheinstetten, Germany), on a Varian 500 MHz INOVA or on a Varian Unity plus 600 (Varian, Palo Alto, CA) NMR spectrometer. Signals are reported in parts per million referenced to CD_2Cl_2. For structural elucidation and NMR signal assignment 2D NMR experiments, such as (H,H)-correlated spectroscopy (H,H-COSY), heteronuclear multiple-quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC), 1D nuclear Overhauser enhancement spectroscopy (NOESY), and total correlation spectroscopy (TOCSY) were performed. Pulse programs for these experiments were taken from the software library.

Chiroptical Methods. UV/vis and circular dichroism (CD) spectra were recorded as methanol solutions in a 0.1 cm cell on a Perkin-Elmer UV/vis Spectrometer Lambda 40 (Perkin-Elmer, Fremont, United States) and a Jasco J-600 spectropolarimeter (Jasco, Gross-Umstadt, Germany), respectively. Specific rotation was determined with a Modular Circular Polarimeter 200 (Anton Paar GmbH, Graz, Austria).

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Known Fusarins. To investigate the rearrangement of fusarin C and to characterize fusarin C analogues formed during fungal growth as well as during storage or isolation, the fungal strain *F. verticillioides* MRC0712 was grown on MYRO medium. After 5 days, the fusarins were extracted from the fungal cultures and further purified by preparative HPLC.[20−22] For preparative HPLC, RP and NP conditions were compared, although it is known that fusarins could isomerize under RP conditions using aqueous solvents. As can be seen from Figure 2, a better

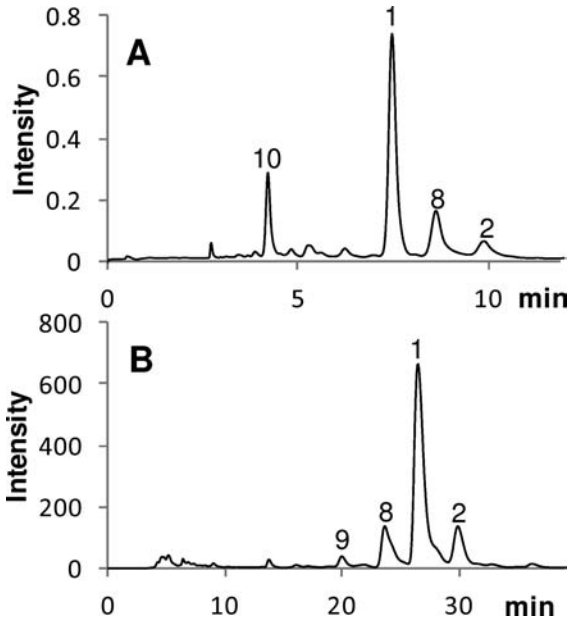

Figure 2. Preparative HPLC-UV chromatograms of the crude fusarin extract. (A) Demonstrates the isolation under NP conditions and (B) under RP conditions (10, open-chain fusarin C; 1, fusarin C; 8, *epi*-fusarin C; 2, fusarin D; and 9, dihydrofusarin C).

separation and peak shape as well as a faster separation of fusarins was obtained with NP.[13] Using the optimized procedure, the following fusarins were isolated, and their structures were elucidated by NMR and MS: fusarin C, fusarin D, and fusarin A and induced by UV light (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C. NMR and exact mass measurements of the isolated compounds are in agreement with literature data.[7,9,11−13,23] Although these compounds are already known, their ^1H and ^{13}C NMR are included in Tables 1 and 2 as these data are needed for the structure elucidation of unknown fusarins. Besides the already known fusarins, the structures of the new metabolites *epi*-fusarin C, open-chain fusarin C, and dihydrofusarin C have been elucidated and will be described in detail in the following sections.

Structure Elucidation of New Metabolites: *epi*-Fusarin C and Open-Chain Fusarin C. NMR data of one new metabolite, the rearrangement product of fusarin C, were assigned as *epi*-fusarin C (Figure 1). The NMR data of *epi*-fusarin C correlate with the data already published for fusarin F[13]. However, in this paper, a different structure assignment was proposed. According to our structure elucidation, we propose the involvement of the pyrrolidone ring, and to prove this, we synthesized 15-methoxy-fusarin C in the next step.

Synthesis of 15-Methoxy-fusarin C. The hydroxy group of the C-15 atom was protected as a methyl ether according to a protocol of Yamaguchi et al.[15] This working group demonstrated that under acidic conditions only the β-methoxy

dx.doi.org/10.1021/jf3009469 | J. Agric. Food Chem. 2012, 60, 5497−5505

derivative is obtained stereoselectively. HPLC analysis of 15-methoxy-fusarin C revealed the formation of only a single peak, which demonstrates the inhibition of the rearrangement. The structure of 15-methoxy-fusarin C was confirmed by NMR (Tables 1 and 2), and NOE experiments proved the stereoselectivity of the synthesis.

Density Functional Calculations of NMR Chemical Shifts. Because there are two possibilities for the rearrangement of fusarin C with an involvement of the 15-hydroxy group, DFT calculations were performed to compare calculated chemical shifts with experimental data. On the one hand, the epoxide group can be shifted from C-13/C-14 to C-14/C-15 as discussed for fusarin F[13], and on the other hand, the stereocenter at C-15 could epimerize as discussed for lucilactaene.[15] The structures used for DFT calculations were simplified as shown in Figure 3. To see the impact of different

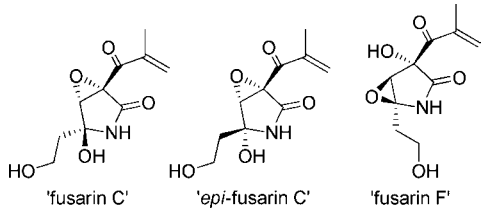

Figure 3. Simplified structures of 'fusarin C', '*epi*-fusarin C', and 'fusarin F' used for DFT calculations.

intramolecular hydrogen bonds on the chemical shifts, conformational isomers were optimized.[19] Finally, the ^{1}H and ^{13}C chemical shifts of all conformers were calculated with Gaussian 03 at same level of theory.[19] Table 3 presents the calculated NMR data of the energetically favored structures of simplified 'fusarin C', '*epi*-fusarin C', and 'fusarin F'.

Table 3. Calculated NMR Data of Simplified Structures of 'Fusarin C', '*epi*-Fusarin C', and 'Fusarin F' (See Figure 3 for Structures)

	'fusarin C'		'*epi*-fusarin C'		'fusarin F'	
C	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
10	136.5	6.4/6.2	130.6	6.4/5.5	128.2	6.1/5.2
11	147.6		146.8		150.6	
12	193.7		198.2		201.9	
13	64.2		65.6		81.2	
14	62.3	3.1	63.1	3.7	64.2	2.9
15	85.7		86.4		71.8	
16		4.4		4.0		6.6
17	167.8		167.5		172.2	
18	37.7	2.0/1.4	40.4	1.3/2.1	30.8	2.5/1.1
19	56.6	3.4/3.3	56.5	3.5/3.5	60.3	4.0/3.8

Because only one hydrogen atom is directly attached to the pyrrolidone ring, little information can be obtained by comparing the calculated ^{1}H NMR spectra. Comparing the calculated ^{13}C NMR of 'fusarin C' (Table 3) with the experimental data of fusarin C in more detail (Table 1), almost no differences are detectable. Hence, it was proven that DFT calculations give very good results for these compounds. Comparing the ^{13}C chemical shift of the isolated *epi*-fusarin C with the calculated '*epi*-fusarin C', they are again in good accordance. However, the calculated NMR data of 'fusarin F' (simplified compound) do not fit to the published results for fusarin F[13] as the difference between the calculated chemical shift of C-13 (81.2 ppm) and the experimental value (64.7 pm) accounts for about 20 ppm. In contrast, the experimental NMR shifts of *epi*-fusarin C correlate to the published NMR data of fusarin F, which raised doubts about the correct structural assignment of fusarin F. Considering a stereoinversion at C-15, the reaction of fusarin C to *epi*-fusarin C would involve an open-chain form as can be seen from the proposed reaction mechanism in Figure 4. The isolation and structure elucidation of an open-chain fusarin C subsequently proved our proposed rearrangement mechanism as shown in the next section.

Isolation and Structure Elucidation of Open-Chain Fusarin C. After storage of a fusarin C stock solution at −80 °C in MeOH, an additional peak was detectable under NP conditions (Figure 5A). A similar chromatogram has already been published by Gelderblom et al.[5] in 1983. In this publication, the peaks were marked P_1, P_3, and P_4, and it was observed that the compounds P_1 and P_4 can be converted to P_3, which was later identified as fusarin C[5]. Comparing the retention times[5] of P_1 and P_4 with our chromatogram (Figure 2A), P_4 can clearly be assigned as *epi*-fusarin C, but the structure of P_1 has never been elucidated. The previous report also stated that P_1 slowly converts not only to P_3 but also to P_4[5]. The isolation of this new compound (P_1) yielded only small amounts, but NMR data unequivocally confirmed the structure of an open-chain fusarin C. The NMR data of the open-chain fusarin C are summarized in Tables 1 and 2. Comparing the chemical shifts of open-chain fusarin C with the NMR data of fusarin C, no differences were detectable for the pentaene chain, but in contrast, the 2-pyrrolidone ring yielded different chemical shifts. First, ^{13}C analysis showed an additional keto group with a chemical shift of 202.7 ppm. This additional keto group was linked to the C-15 atom, proven by HMBC experiments, which revealed long-range couplings of H-18 and H-14 with the C-15 of the additional keto group (Figure 6). In addition, the ^{1}H and ^{13}C signal of 18 are shifted downfield, thus demonstrating the adjacency to the C-15 keto group. Furthermore, two hydrogen atoms are detectable for the amide.

Open-chain fusarin C is only stable under NP conditions. Separating the compound under RP conditions leads to a typical chromatogram of fusarin C and *epi*-fusarin C (Figure 5B).

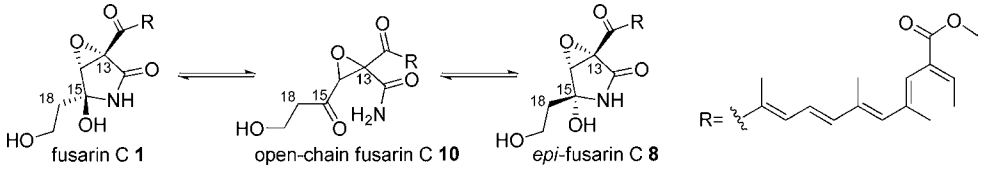

Figure 4. Rearrangement mechanism of fusarin C and *epi*-fusarin C via the open-chain form (for a better comparison of the NMR data, the same numbering was used for all compounds).

dx.doi.org/10.1021/jf3009469 | J. Agric. Food Chem. 2012, 60, 5497−5505

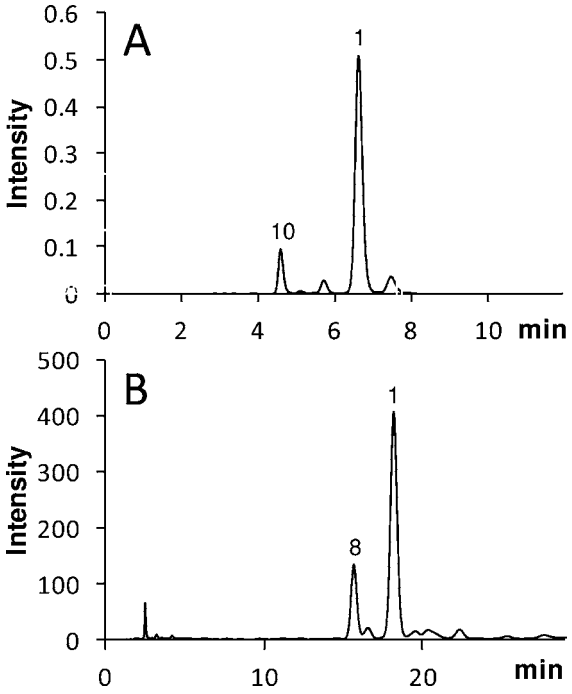

Figure 5. HPLC-UV chromatogram of open-chain fusarin C. The NP chromatogram (A) demonstrates the formation of an additional peak after storage of fusarin C stock solution at −80 °C. (B) Presents open-chain fusarin C under RP conditions forming fusarin C and *epi*-fusarin C (10, open-chain fusarin C; 1, fusarin C; and 8, *epi*-fusarin C).

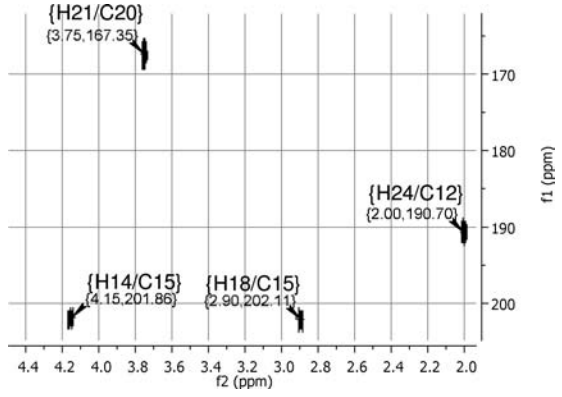

Figure 6. Expanded plot of the HMBC spectrum of open-chain fusarin C. The long-range coupling of H-18 and H-14 with the C-15 keto group demonstrates the open form.

To conclude, the isolation and structure elucidation of open-chain fusarin C unequivocally confirmed the rearrangement mechanism shown in Figure 4. Therefore, the previously published structural assignment of fusarin F is incorrect, since fusarin C epimerizes to *epi*-fusarin C and not to fusarin F.

Structure Elucidation of Dihydrofusarin C. HPLC-DAD and MS/MS analysis of crude extracts revealed a new metabolite, which has a fragmentation pattern very similar to fusarin C (Figure 7). Comparing the product ion spectra of fusarin C and the new metabolite in more detail, most fragments are two mass units higher than the fragments of fusarin C as, for example, *m/z* 267/269, 290/292, 335/337, and 436/438. Exact mass measurements confirmed a molecular formula of $C_{23}H_{31}NO_7$, which is similar to fusarin C but with two additional hydrogen atoms. Therefore, the new metabolite

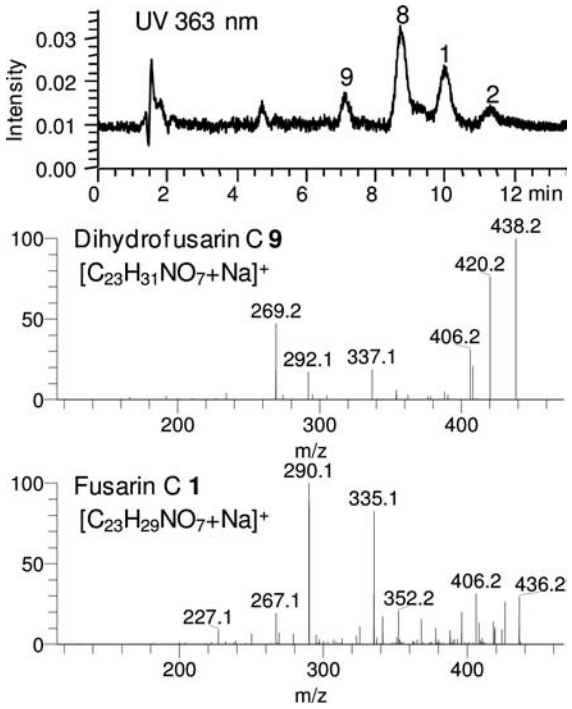

Figure 7. HPLC-UV-FTMS/MS chromatogram of crude fusarin C extract. The chromatogram shows the UV trace. The product ion spectra of dihydrofusarin C (*m/z* 456.18) and fusarin C (*m/z* 454.18) are shown below (1, fusarin C; 8, *epi*-fusarin C; 2, fusarin D; and 9, dihydrofusarin C).

was named dihydrofusarin C. To elucidate its structure and to answer the question at which position the two additional hydrogen atoms are located, reference material was isolated under RP conditions. As the fungal strain produced only minor amounts of this metabolite, it was difficult to obtain sufficient material for ^{13}C NMR analysis. In addition, dihydrofusarin C rearranges under RP conditions like fusarin C, and for this reason, two similar metabolites overlap in the ^1H NMR. To assign the ^1H NMR signals, dihydrofusarin C was methylated at the hydroxyl group at C-15 according to the synthesis of 15-methoxy-fusarin C. The resulting compound was more stable, and ^1H NMR data could easily be measured. The ^1H NMR of the methylated compound was compared to ^1H NMR of the dihydrofusarin C and *epi*-dihydrofusarin C. Tables 1 and 2 summarize the NMR data of dihydrofusarin C, which were similar to fusarin C. To answer the question at which position two additional hydrogens are located, ^1H NMR, TOCSY, and NOE spectra were analyzed. The pentaene chain has the same chemical shifts as fusarin C, which leads to the conclusion that the two additional protons are located at the 2-pyrrolidone ring. ^1H NMR revealed two additional protons with a chemical shift of 4.64 and 4.35 ppm, respectively. Also, the chemical shift of 4.1 ppm, which is typical for the H-14 proton of fusarin C, is missing. Furthermore, TOCSY experiments showed that both protons (4.64 and 4.35) are connected by spin coupling. This clearly shows that the epoxide group is converted into a hydroxy group. Because two protons with different chemical shifts are located at the 2-pyrrolidone ring, the hydroxy group must be located at the C-14 atom. Taking into account that the epoxide group of fusarin C has an (*R*,*R*)-configuration, it is most likely that the carbon atom bearing the hydroxy group of dihydrofusarin C has the same configuration. No significant

dx.doi.org/10.1021/jf3009469 | J. Agric. Food Chem. 2012, 60, 5497−5505

NOEs were detected for H-13 and H-14; therefore, the protons are located *trans* to each other. On the basis of these NMR data, Figure 1 presents the structure of the new metabolite dihydrofusarin C.

Isolation and Structure Elucidation of (*Z*)-Isomers. Another rearrangement of fusarin C is caused by long-wave UV light. Thereby, the pentaene chain of fusarin C rearranges to form (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C.[2,5,9−12,20] To obtain these isomers, a stock solution of fusarin C was irradiated at a wavelength of 366 nm for about 4 h. The elution order of fusarins differs under NP and RP conditions as shown in Figure 8. Tables 1 and 2 summarize the obtained NMR data. The

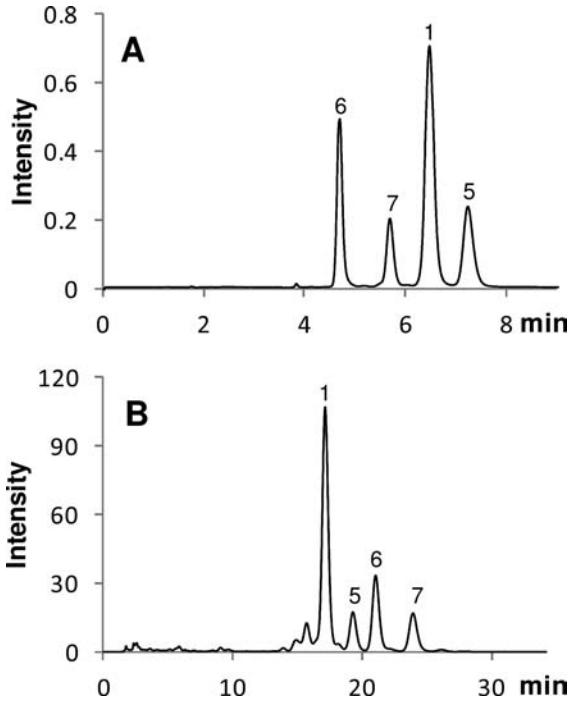

Figure 8. (A) Presents the NP and (B) the RP HPLC-UV chromatogram of fusarin C (1) and its (*Z*)-isomers [5, (10*Z*)-fusarin C; 6, (8*Z*)-fusarin C; and 7, (6*Z*)-fusarin C].

conformational constitution of the pentaene chain was elucidated by NOE experiments. Earlier nuclear Overhauser enhancement experiments indicated that the 2*E*-, 4*E*-, 6*E*-, 8*E*-, and 10*E*-polyene chromophore of fusarin C exists in solution as an equilibrium between two conformers with s-*cis* and s-*trans* topology of the C-5 and C-6 single bond.[11] This topology is also observed for all (*Z*)-isomers. ^{13}C data and complete ^{1}H data of (10*Z*)-fusarin C have not been published before. All further data were in accordance with literature.[9−12]

Product ion spectra of the (*Z*)-isomers revealed the same fragmentation pattern as for fusarin C. Differences were detectable in the relative intensity of the fragments. In addition, the (*Z*)-isomers form an equilibrium between the (*R*)- and the (*S*)-form of the molecule like fusarin C and *epi*-fusarin C. The occurrence of these (*Z*)-isomers in food and feed samples has been shown in a previous report.[1]

CD of Fusarins. To determine the absolute configuration of natural products, CD is the method of choice. Nevertheless, the presence of a chromophore is essential, and the chiral information should be located close to the chromophoric system.[24]

In this study, the CD spectra of fusarin C, *epi*-fusarin C, fusarin D, fusarin A, (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C were measured and are displayed in Figure 9. To differentiate the stereochemistry of fusarin C and *epi*-fusarin C, the CD effect between 200 and 250 nm is crucial because this absorption range reflects the pyrrolidone ring. Because fusarin C and *epi*-fusarin C are epimers, the CD effects are not opposite like the CD effects of enantiomers. Figure 9A shows the difference between fusarin C and *epi*-fusarin C between 200 and 300 nm, confirming the different stereochemistry at the pyrrolidone ring. At the absorption maximum at about 363 nm, which reflects the pentaene chain, CD effects of both epimers are the same, thus demonstrating that the constitutional structure of the pentaene chain remains constant.

Fusarin D shows positive Cotton effects (CE) at 225 and 375 nm and a negative CE at 325 nm. The difference between fusarin C and *epi*-fusarin C is most likely due to a different steric alignment. Fusarin A, in contrast, has only a weak CD effect, which is depicted in Figure 9B. Interestingly, the CD spectra of the (8*Z*)-isomer display negative Cotton effects at 260 and 360 nm and positive CEs at 210 and 315 nm. The spectra of (6*Z*)- and (10*Z*)-fusarin C reveal a positive CE at 210 and 310 nm and a negative CE at 235 nm (Figure 9C). It is known for carotenoids that the introduction of one *cis* double-bond into the polyene chain causes a complete inversion on the CD spectrum.[25] This is presumably the explanation for the different behavior of the (*Z*)-isomers.

In summary, we could elucidate for the first time the rearrangement mechanism of fusarin C and other fusarins by detailed NMR studies, DFT calculations, synthesis of 15-methoxy-derivatives, and the comparison with the NMR data of other fusarins. Fusarin C rearranges to *epi*-fusarin by epimerization of the stereocenter at C-15. As intermediate, an open-chain fusarin C is formed by opening of the pyrrolidone

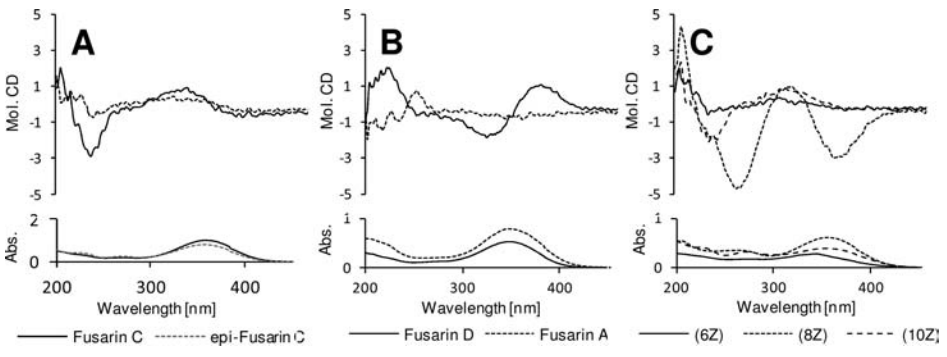

Figure 9. UV and CD spectra of fusarin C, *epi*-fusarin C, fusarin D, fusarin A, and (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C in MeOH.

dx.doi.org/10.1021/jf3009469 | *J. Agric. Food Chem.* 2012, 60, 5497−5505

ring. Both structures were unequivocally identified for the first time. Understanding the rearrangement mechanisms of fusarin C and knowing the exact structures of the rearrangement products is crucial for a substantiated risk assessment and particularly for the evaluation of toxicological studies.

■ ASSOCIATED CONTENT

Supporting Information

NOE data of all isolated compounds and the *x*, *y*, *z* coordinates of the calculated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +49 251 8333391. Fax: +49 251 8333396. E-mail: humpf@uni-muenster.de.

Funding

This project was funded by the Deutsche Forschungsgemeinschaft (DFG) HU 730/9-1.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Wentzel Gelderblom for providing the *F. verticillioides* strain MRC0712. We also thank the International Research Training Group Münster/Germany−Nagoya/Japan for the seminar and practical course "Introduction to Computational Chemistry". We thank Junichiro Yamaguchi, Nagoya University, for helpful discussions.

■ REFERENCES

(1) Kleigrewe, K.; Soehnel, A. C.; Humpf, H. U. A new high-performance liquid chromatography-tandem mass spectrometry method based on dispersive solid phase extraction for the determination of the mycotoxin fusarin C in corn ears and processed corn samples. *J. Agric. Food Chem.* **2011**, *59*, 10470−10476.

(2) Scott, P. M.; Lawrence, G. A.; Matula, T. I. Analysis of toxins of *Fusarium moniliforme*. In *Mycotoxins and Phycotoxins*; Steyn, P. S., Vleggaar, R., Eds.; Elsevier: Amsterdam, The Netherlands, 1985; pp 306−316.

(3) Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O.; van der Merwe, K. J. Natural occurrence of fusarin C, a mutagen produced by *Fusarium moniliforme* in corn. *J. Agric. Food Chem.* **1984**, *32*, 1064−1067.

(4) Thiel, P. G.; Gelderblom, W. C. A.; Marasas, W. F. O.; Nelson, P. E.; Wilson, T. M. Natural occurrence of moniliformin and fusarin C in corn screenings known to be hepatocarcinogenic in rats. *J. Agric. Food Chem.* **1986**, *34*, 773−775.

(5) Gelderblom, W. C. A.; Thiel, P. G.; Van der Merwe, K. J.; Marasas, W. F. O.; Spies, H. S. C. A mutagen produced by *Fusarium moniliforme*. *Toxicon* **1983**, *21*, 467−473.

(6) Cheng, S. J.; Jiang, Y. Z.; Li, M. H.; Lo, H. Z. A mutagenic metabolite produced by *Fusarium moniliforme* isolated from Linxian county, China. *Carcinogenesis* **1985**, *6*, 903−905.

(7) Wiebe, L. A.; Bjeldanes, L. F. Fusarin C, a mutagen from *Fusarium moniliforme* grown on corn. *J. Food Sci.* **1981**, *46*, 1424−1426.

(8) Lu, S. J.; Ronai, Z. A.; Li, M. H.; Jeffrey, A. M. *Fusarium moniliforme* metabolites: Genotoxicity of culture extracts. *Carcinogenesis* **1988**, *9*, 1523−1527.

(9) Barrero, A. F.; Sánchez, J. F.; Oltra, J. E.; Tamayo, N.; Cerdá-Olmedo, E.; Candau, R.; Avalos, J. Fusarin C and (8*Z*)-fusarin-C from *Gibberella fujikuroi*. *Phytochemistry* **1991**, *30*, 2259−2263.

(10) Eilbert, F.; Thines, E.; Arendholz, W. R.; Sterner, O.; Anke, H. Fusarin C, (7*Z*)-fusarin C and (5*Z*)-fusarin C; inhibitors of dihydroxynaphthalene-melanin biosynthesis from *Nectria coccinea* (*Cylindrocarpon* sp.). *J. Antibiot. (Tokyo)* **1997**, *50*, 443−445.

(11) Gelderblom, W. C. A.; Marasas, W. F. O.; Steyn, P. S.; Thiel, P. G.; van der Merwe, K. J.; van Rooyen, P. H.; Vleggaar, R.; Wessels, P. L. Structure elucidation of fusarin C, a mutagen produced by *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun* **1984**, 122−124.

(12) Gelderblom, W. C. A. *The Chemical and Biological Properties of Fusarin C, a Secondary Mutagenic Metabolite Produced by Fusarium moniliforme Sheldon*; University of Stellenbosch: 1986.

(13) Savard, M. E.; Miller, J. D. Characterization of fusarin F, a new fusarin from *Fusarium moniliforme*. *J. Nat. Prod.* **1992**, *55*, 64−70.

(14) Maragos, C. M.; Busman, M.; Plattner, R. D. Development of monoclonal antibodies for the fusarin mycotoxins. *Food Addit. Contam., Part A* **2008**, *25*, 105−114.

(15) Yamaguchi, J.; Kakeya, H.; Uno, T.; Shoji, M.; Osada, H.; Hayashi, Y. Determination by asymmetric total synthesis of the absolute configuration of lucilactaene, a cell-cycle inhibitor in p53-transfected cancer cells. *Angew. Chem.-Int. Ed.* **2005**, *44*, 3110−3115.

(16) Pontecorvo, G.; Roper, J. A.; Hemmons, L. M.; Macdonald, K. D.; Bufton, A. W. J. The genetics of *Aspergillus nidulans*. *Adv. Genet.* **1953**, *5*, 141−238.

(17) Stewart, J. J. Optimization of parameters for semiempirical methods V: modification of NDDO approximations and application to 70 elements. *J. Mol. Model.* **2007**, *13*, 1173−1213.

(18) Stewart, J. J. P. *MOPAC2009*; Stewart Computational Chemistry: Colorado Springs, CO, 2008.

(19) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision E.01; Gaussian, Inc.: Wallingford, CT, 2003.

(20) Jackson, M. A.; Stewart, J. N.; Peterson, R. E.; Slininger, P. J. Fusarin C purification and measurement in submerged cultures of *Fusarium moniliforme* by high-performance liquid-chromatography. *J. Agric. Food Chem.* **1990**, *38*, 1511−1514.

(21) Farber, J. M.; Sanders, G. W. Production of Fusarin C by *Fusarium* spp. *J. Agric. Food Chem.* **1986**, *34*, 963−966.

(22) Brewer, D.; Feicht, A.; Taylor, A.; Keeping, J. W.; Taha, A. A.; Thaller, V. Ovine ill-thrift in Nova Scotia. 9. Production of experimental quantities of isocyanide metabolites of *Trichoderma hamatum*. *Can. J. Microbiol.* **1982**, *28*, 1252−1260.

(23) Gaddamidi, V.; Bjeldanes, L. F.; Shoolery, J. N. Fusarin C: Structure determination by natural abundance 13C-13C coupling and deuterium-induced 13C shifts. *J. Agric. Food Chem.* **1985**, *33*, 652−654.

(24) Snatzke, G. Circular dichroism and optical rotatory dispersion - principles and application to investigation of stereochemistry of natural products. *Angew. Chem.-Int. Ed.* **1968**, *7*, 14−25.

(25) Noack, K.; Thomson, A. J. Conformation and optical-activity of all-*trans*, mono-*cis*, and di-*cis* carotenoids—Temperature-dependent circular-dichroism. *Helv. Chim. Acta* **1979**, *62*, 1902−1921.